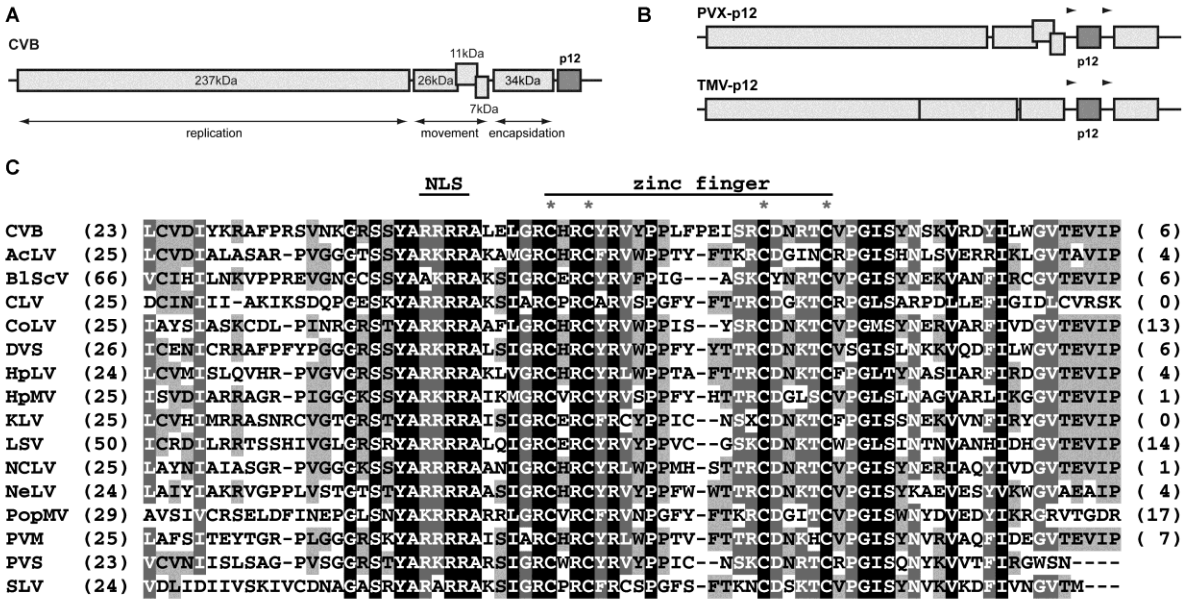
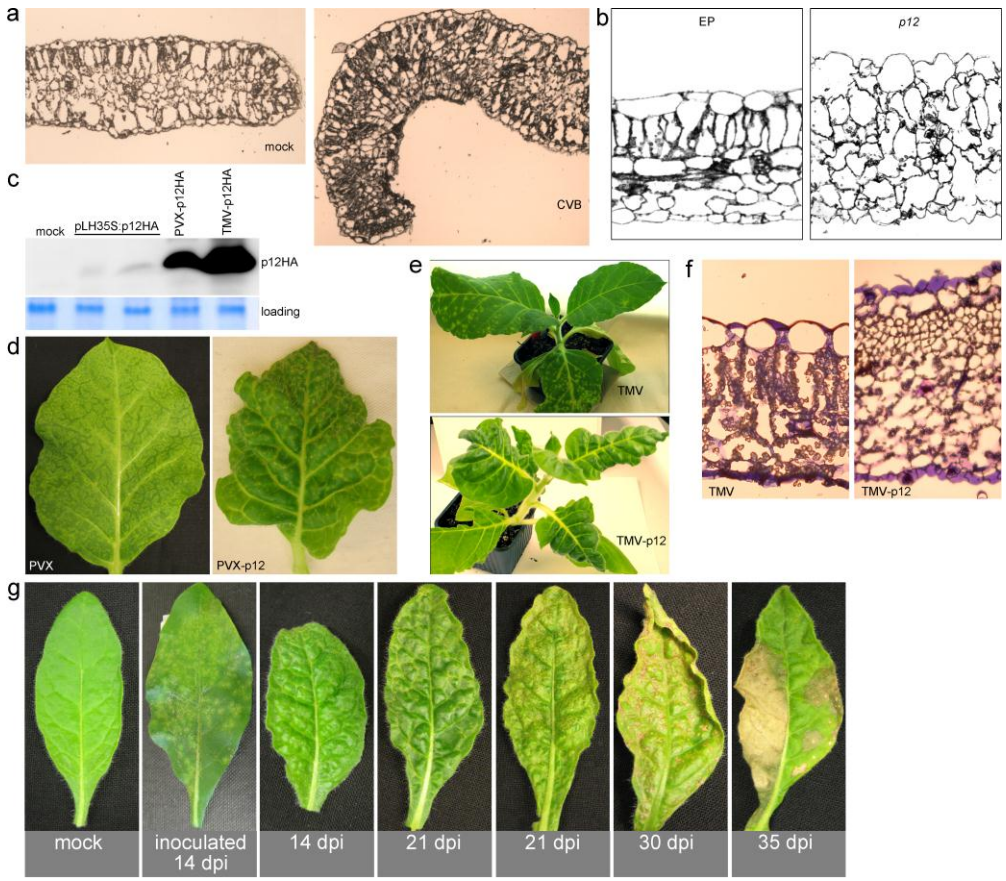


Supplemental Figure 1. Position of *p12* in the virus genome and similarity of the p12 protein to other viral zinc-finger proteins.

(**A**) Genome organization of a typical carlavirus (*Chrysanthemum virus B*). Boxes depict viral genes, molecular masses of the encoded proteins are shown. The double-headed arrows denote functions of the genes during a virus infection cycle.

(**B**) PVX and TMV constructs used in this study. Black arrowheads show positions of duplicated subgenomic RNA promoters. ORF for p12 protein is shaded in dark gray.

(**C**) Sequence alignment of the R**C**xR**C**xRxxPx$_{6-8}$**C**Dxxx**C** zinc-finger domain and adjacent NLS of CRP from 16 carlaviruses. Identical amino acid residues are boxed and shaded grey. Positions of NLS and zinc finger motif are indicated. Asterisks denote cysteine residues involved in zinc finger formation. Numbers of amino acid residues upstream and downstream of the aligned regions are shown in parentheses. The CRPs aligned are from the following caralaviruses: CVB, chrysanthemum virus B (accession number S60150); AcLV, aconitum latent virus (AB051848); BlScV, blueberry scorch virus (L25658); CLV, carnation latent virus (X55897); CoLV, cole latent virus (AY340584); DVS, daphne virus S (AJ620300); HpLV, hop latent virus (AB032469); HpMV, hop mosaic virus (AB051109); KLV, kalanchoe latent virus (AJ293570); LSV, lily symptomless virus (AJ516059); NCLV, narcissus common latent virus (AM158439); NeLV, nerine latent virus (DQ098905); PopMV, poplar mosaic virus (X65102); PVM, potato virus M (D14449); PVS, Potato virus S (AJ863509); SLV, shallot latent virus (AJ292226).



Supplemental Figure 2. p12-mediated hyperplasia induced in Three Plant Species.

(**A**) Cross sections through the tip, the oldest part of the leaf, of chrysanthemum upper leaves from mock inoculated plants and from plants systemically infected with CVB. Note the curling of the CVB-infected leaf.

(**B**) Transient expression by *Agrobacterium* infiltration of CVB *p12* causes hyperplasia 4 dpi relative to empty transferred DNA (T-DNA), EP.

(**C**) Detection of the hemagglutinin-tagged (HA) p12 by Western blotting in samples collected from the *Agrobacterium* infiltrated tobacco leaves ($35S_{pro}$:p12HA construct) and the leaves infected with PVX-p12HA or TMV-p12HA using antisera to the HA-tag.

(**D** and **E**) Appearance of the symptoms induced by empty PVX vector and PVX-p12 (D), and empty TMV vector and TMV-p12 (E) in tobacco. Note severe leaf malformation as compared to mosaic induced by TMV and PVX.

(**F**) Cross sections and light microscopy of upper tobacco leaves infected with TMV and TMV-p12.

(**G**) Foliar symptoms on native tobacco (*Nicotiana occidentalis* ssp. *hesperis*) produced by CVB in upper (unless specified) leaves. Initial symptoms are small necrotic lesions in inoculated leaves 14 dpi and hyperplasia in systemically infected leaves14-21 dpi. Notice the overlap of hyperplasia and necrosis at 21-30 dpi. As the disease progresses, the entire leaf may become necrotic.

a

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                                                          UPA box
                                                          ###################
NtUpp-L  -80 TATTTTTAGCTAAAATTTGG---ATGCCTTATCTCTCTCCTTATGTTTATATAAACCTGACCCTTTAGGACTTTCTCAAACTC -1
NtUpp-S  -80 TATTTTTAGCTAATATTTGG---ATGTCCTATCTCTCTCCTTATGTTTATATAAACCTGACCCTTTGGGACTTTCTCAAACTC -1
CaUPA20  -83 AATTTTAAACTAAAAAAAAGTTTATTTCCTATCTCTCTCTTCATCTTTATATAAACCTGACCCTTTGTGACATTCTGAGACCC -1
```

b

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NtUpp-L -259 GTACTGTGTATGTTTATAATGATAGTACTGTTCTGACATCATTATCTGCTAGCTTAAATTATTTAATATGTTAAA
NtUpp-S -303 GTACTGTGTATATTTATAATGATTGTACTGTTCTGACATCATTATCTGCTAGCTTAAATTATTTAATATGTTAAA

NtUpp-L GGAAAATTATTTAATA-------------------------------------------AGCTTGGCATATTTAATAGC
NtUpp-S TGAAAATTGTTTTATACAAAAAAAAAAAAGAAAAAAAAAGAGAGTTGTATATAGATATAAGCTAAGCATATTTAATAGC

                                                    -103
NtUpp-L TCCCACCTTCCAAAAGCTTGATAAGACCAGTCAAATGCAAAGCAACTGCATGGGTATGAGCTAGAAAATATTTTTAGCTA
NtUpp-S CCCCACCTTCCCAAAGCTTGATTAGACCAGTCAAATACAAAGCAACTGCATGGGAATGAGCTAGAAAGTATTTTTAGCTA

                                                UPA box
NtUpp-L AAATTTGGATGCCTTATCTCTCTCCTTATGTTTATATAAACCTGACCCTTTAGGACTTTCTCAAACTC -1
NtUpp-S ATATTTGGATGTCCTATCTCTCTCCTTATGTTTATATAAACCTGACCCTTTGGGACTTTCTCAAACTC -1
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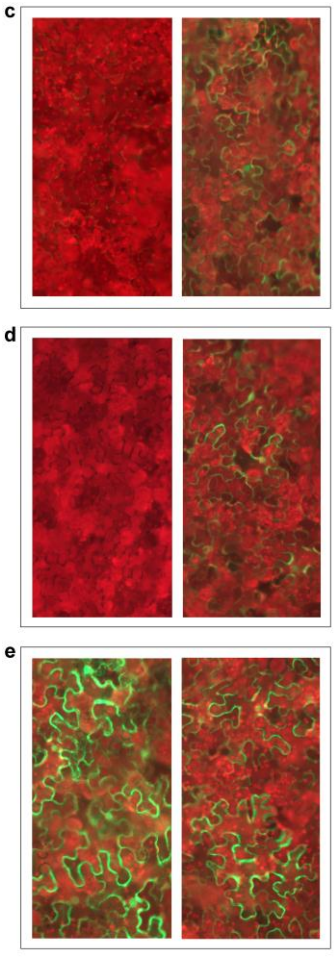
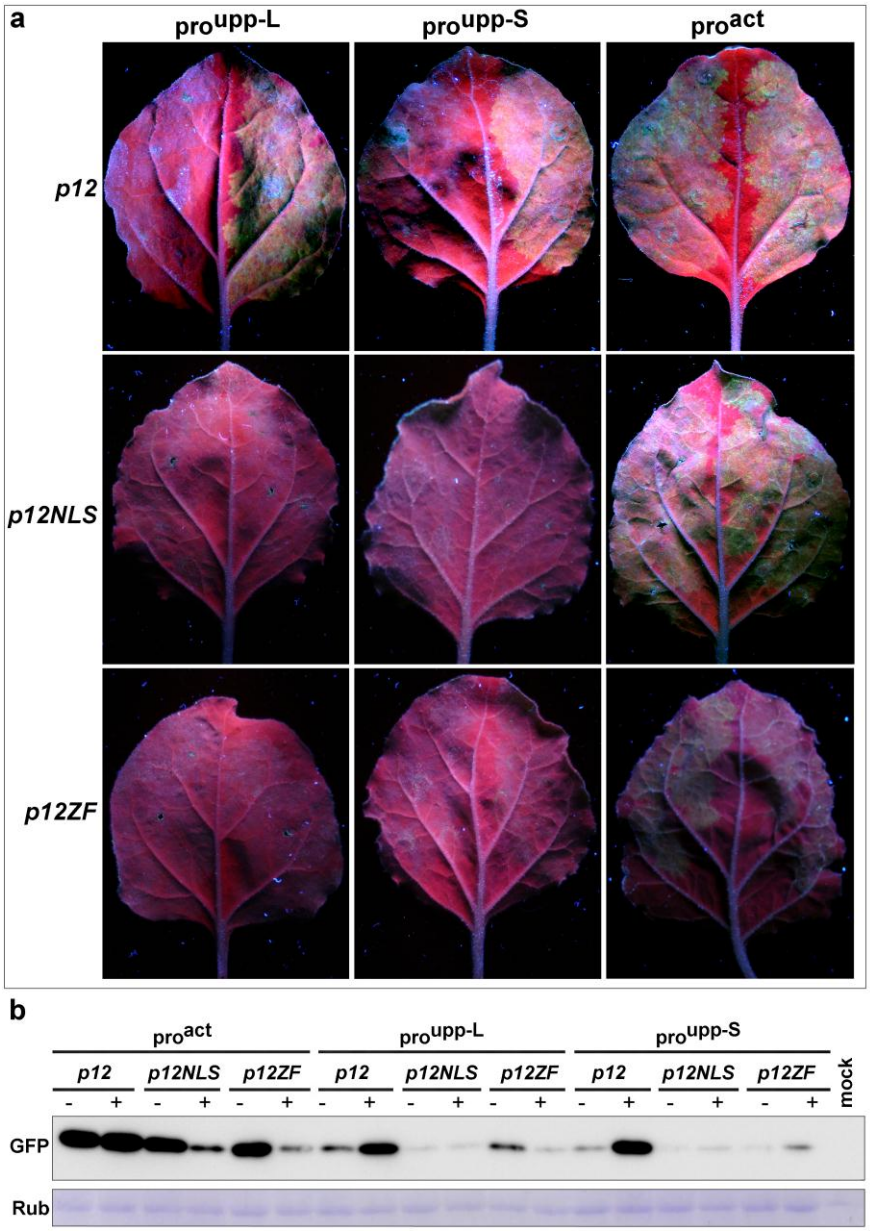


Supplemental Figure 3. Analysis of the *upp* promoter sequences and upp promoter inducibility.

(**A**) Comparison of the sequences surrounding the UPA box in the upp promoters from tobacco and UPA20 promoter from pepper. Note conservation of the UPA box.

(**B**) Sequence comparison of the Nt *upp-L* and Nt *upp-S* promoters. The UPA box is indicated.

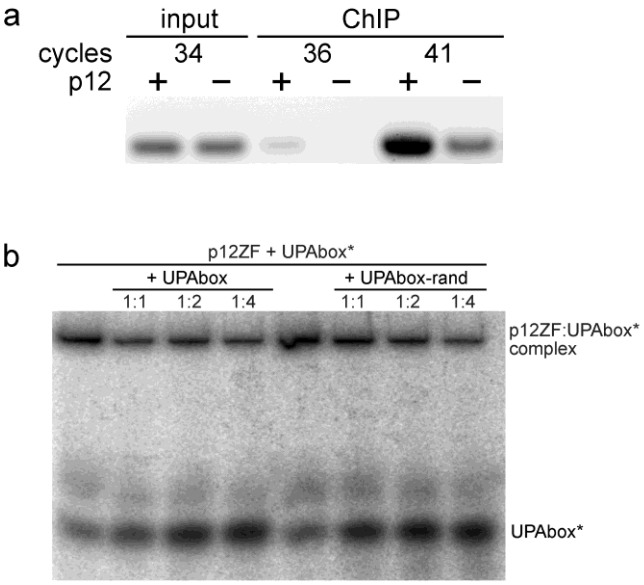
(**C- E**) Fluorescent microscopy images of *N. benthamiana* leaves from the upp promoter inducibility experiments. Analysis of GFP fluorescence 3 dpi shows p12-inducibility of the Nt *upp-L* (C) and Nt *upp-S* (D) promoters. The actin2 promoter construct served as a constitutive promoter control (E). The Nt *upp-L*, Nt *upp-S* and *actin2* promoters were cloned in front of GFP and tested by co-delivery with empty T-DNA (left panels) and p12 (right panels).



Supplemental Figure 4. p12 Mutants with an altered NLS or zinc-finger fail to activate both the Nt *upp-L* and Nt *upp-S* promoters.

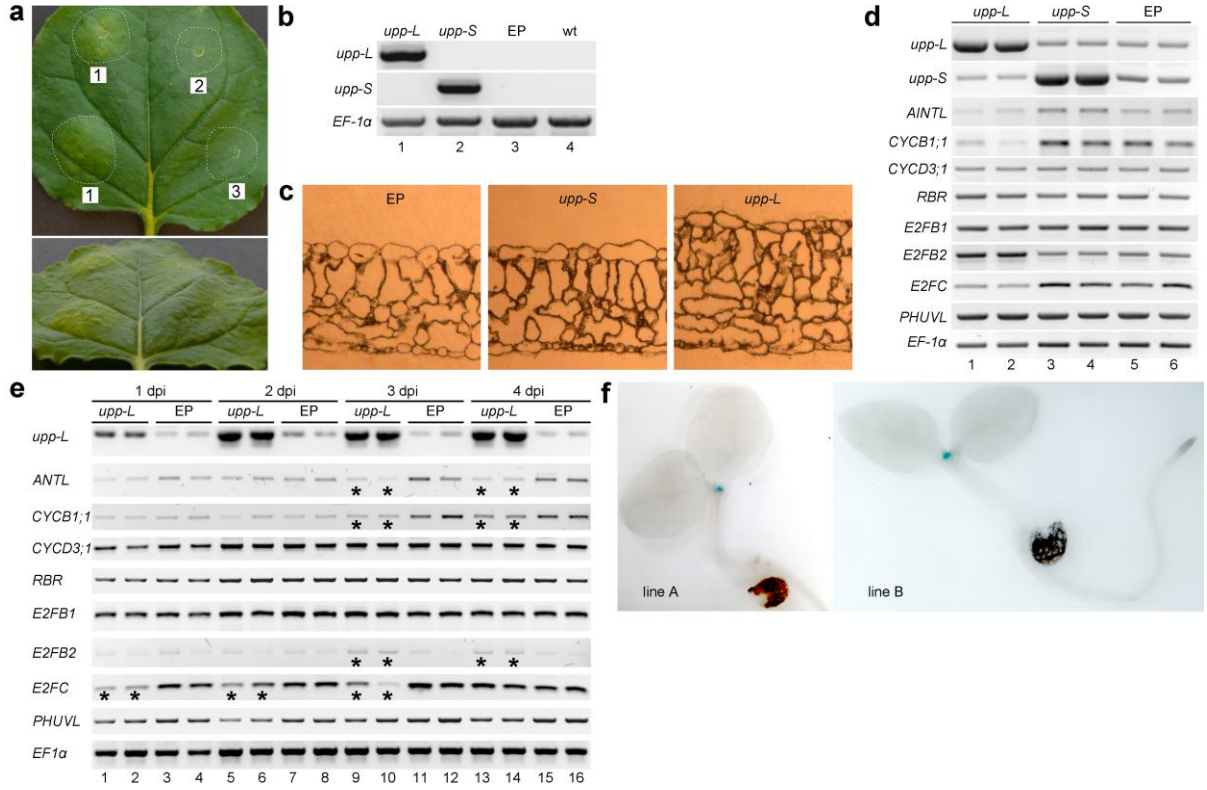
(**A**) Left half of the leaves was *Agrobacterium*-infiltrated for co-delivery of empty T-DNA and promoter:GFP construct as indicated above the panels. Right half of the leaves was infiltrated with promoter:GFP construct (identity of each construct is above the images) and 35Spro:gene construct (identity of each gene or mutant is on the left of panels). p12NLS, the *p12* mutant for NLS. p12ZF, the *p12* mutant for zinc finger.

(**B**) Analysis of GFP expression levels 3dpi through immunoblot. A coomassie blue staining for Rubisco is presented as a loading control.



Supplemental Figure 5. Analysis of p12 association with the *upp* promoters. (**A**) ChIP analysis conducted with HA-specific antibodies on extracts from PVX and PVX-p12HA infected plants. PCR with 34, 36 and 41 cycles was conducted before immunoprecipitation (input) or on immunoprecipitated material (ChIP). The data are from our first experiment, the data from the second experiment are presented in Figure 3B.

(**B**) EMSA of pre-formed complexes of labeled 40-bp $_{pro}$upp fragment containing upa box (UPAbox) and constant amount of p12ZF, a p12 mutant for zinc-finger, (protein:DNA molar ratio of 200:1) incubated with increasing amounts of unlabelled competitor DNA, either UPAbox or UPAbox-rand (same as the UPA box but with randomized upa box sequence), added at the indicated molar ratios to the labeled UPA box. p12ZF is impaired in zinc-dependent DNA binding but retains a weaker non-specific DNA binding activity due to its positively charged NLS (Lukhovitskaya et al., 2009).



Supplemental Figure 6. Ectopic expression of Nt *upp-L* but not Nt *upp-S* causes hyperplasia in leaves, while Nt *upp-L* is normally expressed in the SAM.

(**A**) Transient expression by *Agrobacterium* infiltration of Nt *upp-L* (1) but not Nt *upp-S* (2) causes hyperplasia 4 dpi relative to empty transferred DNA (T-DNA) (3) in *N. benthamiana*, respectively. Little patches were infiltrated as shown by dashed white lines. The picture of the leaf shown in the lower panel was taken from an angle to better visualize hyperplasia.

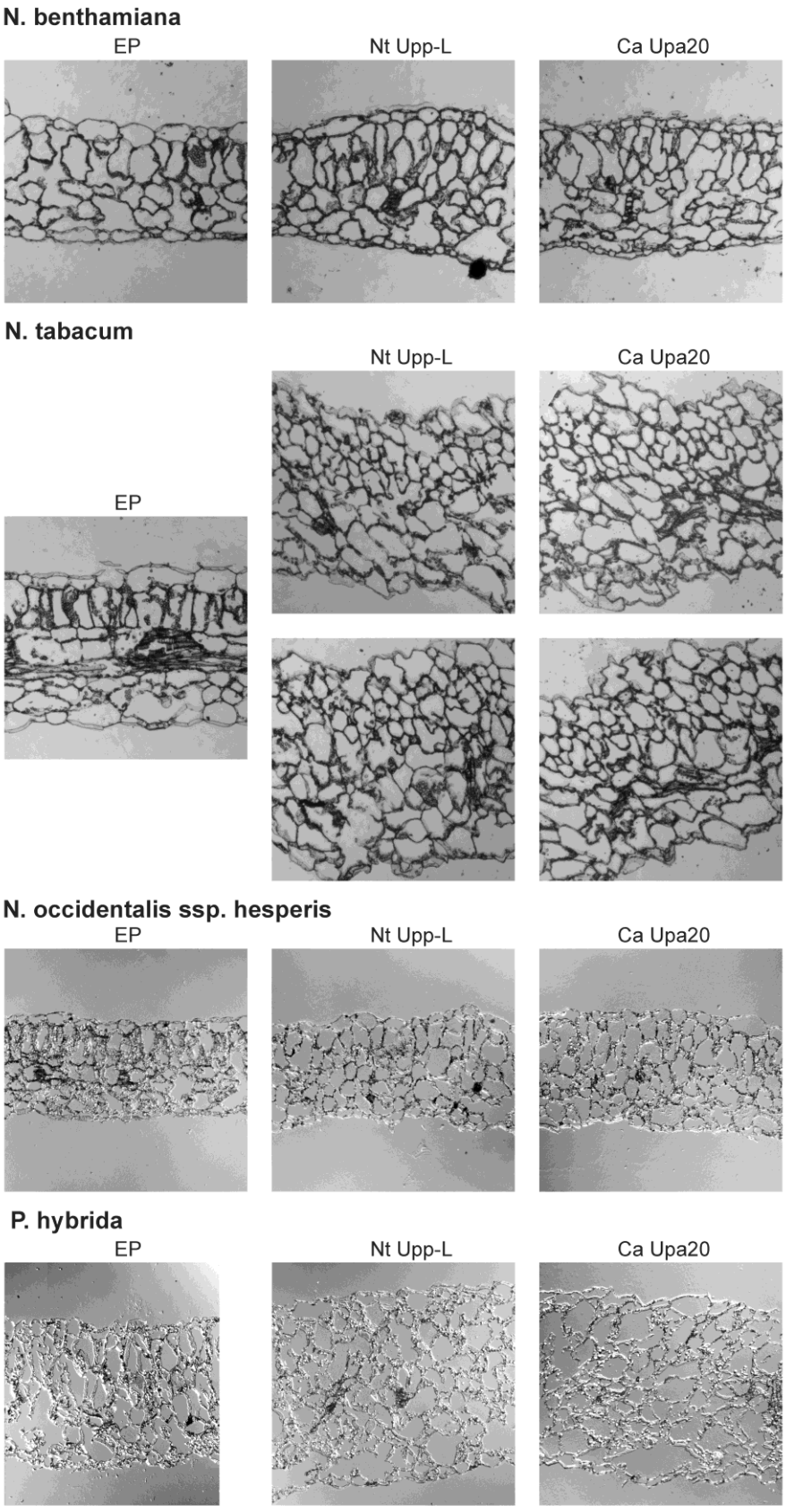
(**B**) RT-PCR on cDNA of *N. benthamiana* leaves *Agrobacterim*-infiltrated for delivery of the Nt *upp-L* and Nt *upp-S* constructs. The constitutively expressed gene for elongation factor 1α (EF1α) served as a normalization control. Note that the primers for Nt *upp-L* and Nt *upp-S* do not detect their orthologs in *N. benthamiana*. EP, empty T-DNA; wt, plant not infiltrated with *Agrobacterium*.

(**C**) Cross sections and light microscopy of *N. benthamiana* leaves 4 dpi with *Agrobacterium*-mediated delivery of Nt *upp-L*, Nt *upp-S* and empty T-DNA. Scale bars correspond to 100 µm.

(**D**) Expression analysis of the genes of the CYCD/Rb/E2F pathway, some leaf patterning genes and Nt *upp-L* and Nt *upp-S* by RT-PCR on cDNA of *Agrobacterium* infiltrated tobacco leaf discs 3dpi. The gene for elongation factor 1α (EF1α) served as a reference gene. EP, empty transferred DNA (T-DNA).

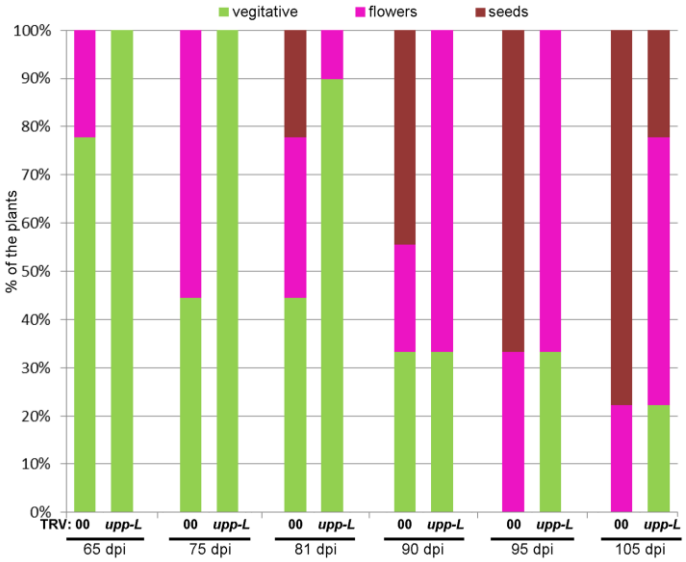
(**E**) Expression analysis of the core cell cycle genes, some leaf patterning genes and Nt *upp-L* by RT-PCR on cDNA of the *Agrobacterium* infiltrated tobacco leaf discs. The upper panel shows strong

overexpression of Nt *upp-L* upon *Agrobacterium*-mediated delivery. Of the eight other genes tested, the transcript levels of one of the Nt *E2F* genes were elevated (indicated with asterisks), and Nt *ANTL*, Nt *CYCB1;1* and Nt *E2FC* genes were downregulated (indicated with asterisks) in the Nt *upp-L*-overexpressing leaf areas. Lanes 1 and 2, 3 and 4, 5and 6 etc. represent two of four plant replicates for each treatment, respectively. The experiment was repeated twice with similar results. The gene for EF1α served as a reference gene. EP, empty transferred DNA (T-DNA).
(**F**) Tissue specific expression of GUS under the control of the Nt *upp-L* promoter. Images show one-week old plants of two transgenic lines, in each case transformed with the *upp-L*pro:*GUS* construct. GUS activity was visualized by treatment with X-GlcA (5-bromo-4-chloro-3-indolyl-b-D-glucuronic acid).


N. benthamiana
EP
Nt Upp-L
Ca Upa20
N. tabacum
Nt Upp-L
Ca Upa20
EP
N. occidentalis ssp. hesperis
EP
Nt Upp-L
Ca Upa20
P. hybrida
EP
Nt Upp-L
Ca Upa20

Supplemental Figure 7. Hyperplasia induction in four plant species by Nt *upp-L* and Ca *UPA20*.

Cross sections and light microscopy of *N. benthamiana*, *N. tabacum*, *N. occidentalis* ssp. *hesperis* and *Petunia hybrida* leaves with the Agrobacterium-mediated delivery of Nt *upp-L*, Ca *UPA20* and empty T-DNA 5 dpi. Previously it was shown that the Xanthomonas pathogenic AvrBs3 TALe induces the UPA20 TF in infected pepper tissue (Key et al., 2007). The phenotypes associated with the Ca *UPA20* induction included cell expansion, reduced starch and increased cellulose accumulation in cell walls. In contrast, our data demonstrated that Nt *upp-L* induced cell proliferation and is expressed in the SAM (Figure 4B; see Supplemental Figure 6C and 6F). It is possible that different members of the UPA/upp-like TFs (Figure 1H) mediate both cell proliferation and cell expansion. To address this question Ca *UPA20* and Nt *upp-L* were individually expressed in *Solanaceae* species by *Agrobacterium* infiltration and tissue morphology was analyzed. Surprisingly, in four independent experiments in four plant species *Agrobacterium*-mediated Ca UPA20 overexpression induced hyperplasia similar to the phenotypes observed upon Nt *upp-L* overexpression. These results differ from the previous report on Ca *UPA20* (Key et al., 2007), but are consistent with earlier reports of the same research group showing e.g. the avrBs3–mediated hyperplasia of *N. clevelandii* tissue (Marois et al., 2002).



Supplemental Figure 8. Delay in vegetative to floral transition of tobacco plants silenced for *upp-L*.

The experiments were repeated twice with similar results (n=18).

Supplemental Table 1. *Upp*/*UPA*-like genes used in the phylogenetic analysis.

Abbreviation	**Binomial name (common name)**	**Accession number**
At	*Arabidopsis thaliana* (thale cress)	At5g50915 (bHLH137), At1g59640 (BIGPETALp)
Bd	*Brachypodium distachyon* (purple false brome)	XP003574844
Ca	*Capsicum annuum* (pepper)	ABW22630
Cs	Citrus sinensis (sweet orange)	ABW97699
Gm	*Glycine max* (soya bean)	XP003526933 XP003522447
Lj	*Lotus japonicus*	ACN21645
Md	*Malus domestica* (apple tree)	EG631304
Mt	*Medicago truncatula* (barrel clover)	AET05042
Nh	*Nicotiana occidentalis* ssp. *hesperis* (native tobacco)	HE653926 *
Nt	*Nicotiana tabacum* cv. Samsun nn (cultivated tobacco)	HE653924 (*upp-L*)* HE653925 (*upp-S*)*
Pt	*Populus trichocarpa* (western balsam poplar)	XP002322296
Rc	*Ricinus communis* (castor bean)	XP002511110
Sb	*Sorghum bicolor* (sorghum)	XP002444680
Sl	*Solanum lycopersicum* (tomato)	AW034575
St	*Solanum tuberosum* (potato)	retrieved from http://solgenomics.net/tools/blast/index.pl
Tg	*Tulipa gesneriana* (tulip)	AAD56411
Vv	*Vitis vinifera* (grape)	XP002284464
Zm	*Zea mays* (maize)	ACG40967

* this study

Supplemental Table 2. *N. tabacum* genes involved in early leaf development and the CYCD/Rb/E2F pathway of the cell cycle used in our analysis.

N. tabacum **gene**	**Accession number**	**Reference**
NtANTL	AY461432	Rieu *et al*, 2005
NtCYCB1;1	Z37978	Qin *et al*, 1995
NtCYCD3;1	AJ011893	Sorrell *et al*, 1999
NtE2FB1	AB025347	Sekine *et al*, 1999
NtE2FB2	HE653923	this study
NtE2FC	HE653922	this study
NtPHAVOLUTA-like HD-ZIPIII	TC82772; AAS66760	McHale and Koning, 2004
NtRBR	AB015221	Uemukai *et al*, 2005

Supplemental Table 3. List of PCR primers.

Primer name	5' primer sequence 3'	Used for
P12Age-FW	CAACCGGTATGGATGTGATTGTG	Cloning TMV-p12
P12Xho-REV	CACTCGAGCATGGTCGAGCCTCC	Cloning TMV-p12
Nt-upa20-RACE3	GCACTCATGGTCAGACCTGACCAGAG	3'RACE *upp-L* and *upp-S*
Nt-upa20-RACE-5	AATAACTTCAGCTGCCTGCGATGTAATG	5'RACE *Nt upp-L, Nt upp-S* and *Nh upp-L,* RT-PCR *Nt upp-L*, *Nt upp-S* and *Nh upp-L*
Upp-Nco-FW	AACCATGGCATCACTTTCTTGAATCCTTCC	cloning *Nh upp-L* mRNA, RT-PCR *Nh upp-L*
Upp-Apa-gene-REV	AAGGGCCCAAATATCCATTAATACTAGTGGTAAA	cloning *Nh upp-L* mRNA
Nt-UppL-qFW-new	GATGACAACAAAAAAGAGGAAAAGAA	RT-PCR
Nt-UppS-qPCR-FW	GAGAAAAAGGGAAAAGAGGAGAAG	RT-PCR
Nt-Upp-qFW3	ATCAAGAAATCAGTGGAAGCC	qRT-PCR *upp-L* and *upp-S*; ChIP fragment 2
Nt-UppL-qR3	TTCCGCCAGTTGACTCTTTG	qRT-PCR *upp-L*
Nt-UppS-qR	GCTTTCTTATTCCGCCAGTTGAC	qRT-PCR *upp-S*
uppL-genomeWalker-rev	ATGGTAACAGAGGCAGTAGTCAAGAGAGAG	*upp-L* genome walking
uppS-genomeWalker-rev	CAATGGGAATTATTTATGGCGAACAATT	*upp-S* genome walking
uppS-genomeWalker-R2	ATTTCCTCAAACATGAAGGGCAACCGCAAG	*upp-S* genome walking
UppL-1359-rev	AGATGTTAAGTAGGGCAGGG	sequencing
UppL-2514-seq-rev	GATGTAGTGTAGGGCAGGG	sequencing
Ca-F0R0-Mun-P	CACAATTGCGCAGGTTCGAATTCCCAATCCAAC	Cloning of $_{pro}$UPA20:*GFP*
Ca-F0R0-Mun-M	CACAATTGCTCGAGCTTTTTCAAGTTTATGATTTGCTTTG	Cloning of $_{pro}$UPA20:*GFP*
Ca-UPA20-Nco-P	CACCATGGGCACCATGTCTACTTTTTCATCATACC	Cloning of *CaUPA20* ORF
UPA20-Xba-M	CATCTAGATTAATGGAAAGAACAAAAGTTGTTG	Cloning of *CaUPA20* ORF
ACT2-prom- Mun-P	CACAATTGATTATGTAAAAGTGCATCAATC	Cloning of $_{pro}$ACT:*GFP*
ACT2-prom Mun-M	CACAATTGCTCGAGTTTATGAGCTGCAAACACACAAAAAG	Cloning of $_{pro}$ACT:*GFP*
Nt-upp-103nt-EcoRI-FW	AAGAATTCGGCATGGGTATGAGCTAG	Cloning of L1U2 and S1U2; ChIP fragment 1
Nt-upa20-103nt-Xho-Rev	AACTCGAGTTTGAGAAAGTCCTAAAG	Cloning of L1U2 and S1U2; ChIP fragment 1
Nt-uppL-prom-EcoR-left	AAGAATTCTATAAAGGTGACGTGACGAATACGT	cloning of L0U0, L0U1 and L0U2
Nt-uppL-gene-EcoRI-FW	ATGAATTCTCATTTAGATAACTGGCTTGAATCATC	cloning of L0U0, L0U1 and L0U2
Nt-UppLS-EcoRI-	TTGAATTCAAACAACAAWAAWAATATCMATT	cloning of L0U1 and

REV		S0U1
Nt-UppLS-NcoI-FW	AACCATGGCATCACTTTCTTGAATCCTTCC	cloning of the *upp-L* and *upp-S* ORFs
Nt-UppS-XbaI-gene-REV	AATCTAGAAAGAAACAACAATAATAATATCAATTACTAG	cloning of the *upp-S* ORF
Nt-UppL-Xba-gene-REV	AATCTAGAAAATATCCATTAATACTAGTGGTAAA	cloning of the *upp-L* ORF
uppLS-Xho103-right	AACTCGAGCAGTTGCTTTGYATTTGACTGGTC	cloning of L0U2 and S0U2
uppL-XhoI-right-FL	AACTCGAGTTTGAGAAAGTCCTAAAGGGTCA	cloning of L0U1
uppLS-EcoR-NO-box-F	AAGAATTCRGGACTTTCTCAAACTCATTATAATATTTA	cloning of L2U3 and S2U3
uppL-5UTR-right-NcoI	AACCATGGCTTTTTTTTTACAAATAATTCTTCTTTATTAG	cloning of L0U0 and L2U3
upaS-5UTR-right-NcoI	AACCATGGCTTTTTTTAAAAAAAAAATTCTTCTTTATTAG	cloning of S0U0 and S2U3
Nt-Upp-XhoI-FW	GACTCGAGCAGAGTTTGAGTGGATTGGGA	cloning of TRV:*upp-L*
Nt-Upp-EcoRI-R	AAGAATTCAAACAACAAAAAAAATATCCATT	cloning of TRV:*upp-L*, ChIP fragment2
NtUPA20-fw	TGCCAAATATGCAGCAAGCTA	RT-PCR *upp-L* and *upp-S*
NtUPA20-rev	CAGCTGCCTGCGATGTAATGT	RT-PCR *upp-L* and *upp-S*
Nt-EF1alpha-fw	GGCCCAACACTTCTTGATGC	qRT-PCR
Nt-EF1alpha-rev	GGGCCTCTTGGGCTCATTAA	qRT-PCR
Nt-Beta-actin-fw	CCCCTTTCAAAACAAGAACGC	qRT-PCR
Nt-Beta-actin-rev	GTTATTGTTGGCGATGGCCT	qRT-PCR
Nt-cdc2-fw	AAATGCTCCGGTTGGATCC	qRT-PCR
Nt-cdc2-rev	CAAGGGCATTCCTGGCAGT	qRT-PCR
Nt-ANT-F	TGCAGCAGCCACAGAAGTAGC	RT-PCR
Nt-ANT-R	GACAATGCATGGGAGAATAATAGC	RT-PCR
Nt-CYCD3-1-F	CTCTTCACACCTCCCACAACACA	RT-PCR
Nt-CYCD3-1-R	GGCAGTCAAAGCAGAGAAACCAT	RT-PCR
Nt-RBR-F	CGTTTTGGCTGGTTGCTATTTCTT	RT-PCR
Nt-RBR-R	CACCCTTGTTCTGTATTGCATCACT	RT-PCR
Nt-E2FB1-F	TACCACCGCTTCTCTACTGACCCA	RT-PCR
Nt-E2FB1-R	GCGCCTTTTCTGCACCTCTAAT	RT-PCR
Nt-RB_F1	TGGTCCAACATTAAGCAATCTGTACG	qRT-PCR
Nt-RB_R1	AAAAGGCTCAAATGCACGAAGTTG	qRT-PCR
Nt_E2Fb_F5	GTCTGGAAAAGCTGGAAACAC	qRT-PCR
Nt_E2Fb_R5	GGGAGCTATCATATCGACAAGG	qRT-PCR
Nt_E2Fc_F5	TTAGCTCCACTTCATCTAATGTCTC	qRT-PCR
Nt_E2Fc_R5	TCGTCTTACAAACTCCATATCCAC	qRT-PCR
Nt_AINT_F5	GTAGTGGATTCTCAAGAGGTGC	qRT-PCR
Nt_AINT_R5	TGTGCTGAAAGTCCCAAGATAG	qRT-PCR
Nt_CYCB1_F2	GCCTGAGAGCCTTTACCTTAC	qRT-PCR
Nt_CYCB1_R2	TCT GGTGCCCAAATCTCTTC	qRT-PCR
Nt-E2F51-seqRACE3	GTTTTCCACAGACCCGGTTCATTGTTCA	3'RACE *NtE2FB2*, RT-PCR
Nt-E2FB2-uni-R	GAGTTCAAATGGCTGTACAGAGGATTT	*5'RACE NtE2FB2,* RT-PCR
Nt-E2Fc-F	AGCTCGGAATTTATTTGCCTCGTCTACA	3'RACE *NtE2FC,* RT-PCR

Nt-E2Fc-R	TCGGCCTCCTGAAGCAAACTAATGAAT	5'RACE *NtE2FC,* RT-PCR
Nt-CYCB1-F	CCGATGGAAGAAATAGGCGTGCT	RT-PCR
Nt-CYCB1-R	TGGTAATTGTAAGGTAAAGGCTCTCAGG	RT-PCR
Nt-PHAVO-F	ACAAGAAACCAGTGGGGAAATCC	RT-PCR
Nt-PHAVO-R	CCACAGTCTGAGCTAGGGGTAAAATG	RT-PCR
Nt-EF1alpha-pos	ATGGGTAAAGAGAAGTTTCAC	RT-PCR
Nt-EF1alpha-neg	CACGATTTCATCATACCTAGC	RT-PCR
UPAbox F	CTCCTTATGTTTATATAAACCTGACCCTTTAGGACTTTCT	EMSA
UPAbox R	AGAAAGTCCTAAAGGGTCAGGTTTATATAAACATAAGGAG	EMSA
UPAbox-rand F	CTCCTTATGATCTCAAGATTTTCTACTATCAGGACTTTCT	EMSA
UPAbox-rand R	AGAAAGTCCTGATAGTAGAAAATCTTGAGATCATAAGGAG	EMSA

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